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A randomized, controlled exposure study in adult smokers of full flavor Marlboro cigarettes switching to Marlboro Lights or Marlboro Ultra Lights cigarettes

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ABSTRACT

Rationale. To date no state-of-the-art clinical study has been conducted to address the question as to whether switching to lower tar cigarettes reduces exposure to smoke constituents in humans. *Methods.* Randomized, controlled, forced switching study in 225 adult smokers of full flavor Marlboro (MFF) cigarettes for 8 days with a 24-week follow-up. Subjects smoked MFF (a 15-mg Federal Trade Commission (FTC) tar cigarette) at baseline and were randomized to smoke 11-mg Marlboro Lights (ML) or 6-mg Marlboro Ultra Lights (MUL) cigarettes. Biomarkers of exposure to nicotine, 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), pyrene, CO, benzene, acrolein, and mutagenic substances were measured. *Results.* In the short-term phase, switching from MFF to ML showed statistically significant decreases in nicotine exposure (−13%) and non-significant increases in CO exposure (+6%), while switching from MFF to MUL showed statistically significant decreases in nicotine (−27%) and CO (−13%) exposure. Both nicotine and CO biomarkers trended similarly in the 24-week follow-up as in the short-term phase. The other biomarkers of cigarette smoke constituents followed the same trend as nicotine at the end of the 24-week follow-up. *Conclusions.* Switching smokers to lower FTC tar yield cigarettes, on average, reduces nicotine and other biomarkers considered surrogates of tar exposure.

1. Introduction

The tar, nicotine and carbon monoxide delivery yields of cigarettes marketed in the US are determined with smoking machines according to the Federal Trade Commission (FTC) standard protocol: 35 ml puff volume, 2 second puff duration, 1 puff/minute frequency (Bradford et al., 1936; Pillsbury et al., 1969). There is a wide range in the tar and nicotine yields of the different cigarettes available on the market, e.g. 1.5–17 mg tar per cigarette (Counts et al., 2006). Smoking machine yields however do not represent the wide range of human smoking behavior and therefore cannot predict the human exposure to smoke constituents. As the FTC itself recognized, the machine-generated yields simply indicate the relative yield of different cigarette brands according to a convention of analytical standards, but not actual smoking conditions of humans (FTC, 1967; Gori and Lynch, 1985). For accurate determination of human exposure to smoke constituents, it is necessary to conduct well-designed clinical studies in an adequate number of adult smokers and to measure the appropriate biomarkers of exposure to tobacco smoke constituents (Institute of Medicine, 2001).

Many human studies, mostly observational and only a few interventional, (Benowitz, 2001 and references therein; Benowitz et al., 2005; Hecht et al., 2005), have compared exposure levels in smokers to cigarettes with different tar yields. In some of these studies smokers were forced to switch from their usual brands to higher or lower tar yield cigarettes while the cross-sectional studies compared exposure in smokers smoking different tar yield cigarettes as their regular brand. On only one occasion was spontaneous brand switching used to examine exposure (Lynch and Benowitz, 1987). Many of the switching studies included only a few subjects (less than 15 subjects), making it difficult to draw generalized conclusions from the results. Nicotine and carbon monoxide (CO) have been the most widely studied biomarkers to quantify human exposure to cigarette smoke (Benowitz, 1996; Benowitz, 1999). However, in the various clinical studies the nicotine exposure was measured using different sampling methods i.e. 24-h urine collection, spot urine collected at a single time point, single blood cotinine measurements at different times during the day, or cotinine in saliva (Jarvis et al., 2001). The biomarker data show high inter-subject variability and conflicting exposure results on the effects of switching to lower tar cigarettes have been reported. (Benowitz, 2001 and references therein; Benowitz et al., 2005; Hecht et al., 2005). Based on the above mentioned limitations, it is difficult to draw conclusions as to whether reducing tar and nicotine yields in cigarettes results in reduced exposure in human smokers.

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Cigarette mainstream smoke is a complex aerosol composed of a mixture of combustion gases and semi-volatile compounds, often referred to as gas/vapor phase (GVP) and minute liquid droplets representing the particulate or "tar" phase (PP) (Benowitz, 2001). Cigarette smoke contains more than 4800 chemical constituents (Green and Rodgman, 1996). The measurement of biomarkers of exposure in urine or blood can provide quantitative estimates of the uptake of relevant smoke constituents (Institute of Medicine, 2001; Hatsukami et al., 2006; Hecht, 2002). These biomarkers also reflect the exposure in relation to cigarette smoking behavior, and to absorption, distribution, metabolism and elimination of relevant smoke constituents. It is impossible to determine exposure to all smoke constituents. It is however necessary to measure multiple biomarkers of particulate and gas/vapor phase smoke constituents, as the amount absorbed is different for the different smoke constituents depending on their physicochemical properties (Baker and Dixon, 2006; Feng et al., 2007a; Moldoveanu and StCharles, 2007). Nicotine (PP) exposure is best assessed by measuring nicotine and its five major metabolites in urine, nicotine-*N*-glucuronide, cotinine, cotinine-*N*-glucuronide, *trans*-3′-hydroxycotinine, and *trans*-3′-hydroxycotinine-*O*-glucuronide, the molar sum calculated as nicotine equivalents [NE], which reflects 90–95% of the nicotine absorbed (Feng et al., 2007b). Plasma cotinine has also been used as a biomarker for nicotine exposure (Benowitz, 2001). 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone, NNK (PP), is a tobacco-specific nitrosamine and a suspected carcinogen in humans. Exposure to NNK can be assessed by measurements of its free and conjugated metabolites 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (total NNAL) in urine (Hecht, 2002). Urinary total 1-hydroxypyrene (1-OHP), free and conjugated metabolites of pyrene (PP), has been used as a surrogate for polycyclic aromatic hydrocarbon (PAH) exposure (Feng et al., 2006; Hecht, 2002; Scherer et al., 2000). Urine mutagenicity assay with *Salmonella typhimurium* has been widely used to detect exposure to mutagens (Hatsukami et al., 2006; Roethig et al., 2005). Carboxyhemoglobin (COHb) has been used as a biomarker of CO exposure (Benowitz et al., 1986; Roethig et al., 2005; Roethig et al., 2007). *S*-phenylmercapturic acid (*S*-PMA) is a metabolite of benzene (GVP), classified as a Group I carcinogen by the International Agency for Research on Cancer (IARC). Urinary *S*-PMA has been widely used in environmental, occupational, and smoking-related exposure studies (Feng et al., 2006; Hecht, 2002; Qu et al., 2000). Acrolein (GVP), an airway irritant, is an aldehyde and present in cigarette smoke, environment and foods. Its urinary metabolite, 3-hydroxypropylmercapturic acid (3-HPMA), has been used as a biomarker for smoking-related acrolein exposure (Mascher et al., 2001; Roethig et al., 2007).

The purpose of the present study was to evaluate short-term and long-term changes in these biomarkers of exposure in adult smokers switching from full flavor Marlboro (MFF) cigarettes to Marlboro Lights (ML) or Marlboro Ultra Lights (MUL) cigarettes.

2. Materials and methods

2.1. Subjects

This study was performed in accordance with Good Clinical Practice (GCP) and the Declaration of Helsinki criteria (World Medical Association, 2000). The protocol and informed consent form were reviewed and approved by the MDS Pharma Services Institutional Review Board (IRB). All volunteers provided written informed consent before enrolling in the study, were paid for participating, and were free to discontinue the study at any time for any reason.

Healthy male and female adults who reported smoking between 10 and 30 full flavor (15-mg tar) cigarettes daily were recruited from the general population in Lincoln, NE and Phoenix, AZ through IRB-approved local newspaper advertisements. A total of 257 subjects were admitted to the clinic on Day 3 (check-in) to ensure that at least 225 healthy adult male and female smokers were randomized into one of three groups (75 subjects per group).

Concomitant medications were generally permitted in stable doses to treat an investigator-approved condition (e.g. hypertension or seasonal rhinitis). Additionally, hormonal contraceptives, hormone replacement therapy, occasional use of over-the-counter analgesics, antacids, H_2-antagonists, antihistamines, and nasal decongestants were allowed. Subjects with clinically significant diseases, health conditions or abnormal laboratory results, pregnant or lactating females and subjects who required anti-diabetic or insulin therapy, bronchodilators, or antibiotic therapy for an acute infection were excluded.

2.2. Test products

The products used in this study were full flavor Marlboro (MFF), Marlboro Lights (ML) and Marlboro Ultra Lights (MUL) cigarettes. They were analyzed for tar and nicotine delivery in mainstream smoke using the standard FTC smoking machine protocol (Bradford et al., 1936; Pillsbury et al., 1969). The tested cigarettes delivered 15 mg tar and 1.1 mg nicotine (MFF), 11 mg tar and 0.8 mg nicotine (ML) and 6 mg tar and 0.5 mg nicotine (MUL). Additional information on other smoke constituents of these three brands is presented in Table 1. The cigarettes used in this study all had cellulose acetate filters and contained American-blend tobacco comprising Bright, Burley, Oriental and reconstituted tobaccos. The circumference of all cigarettes was about 24.8 mm. The cigarette lengths were 79 mm for MFF and 83 mm for ML and MUL cigarettes; the tobacco weights were 0.694, 0.653 and 0.613 g for MFF, ML and MUL, respectively. Ventilation was 10, 17 and 43% for MFF, ML and MUL, respectively. These product characteristics apply to cigarettes manufactured in 2003 when the study was conducted. All test cigarettes used during the short- and long-term phases of the study were provided by Philip Morris USA.

2.3. Study design and conduct

The study included two phases: (1) a short-term phase with a randomized, controlled, open-label, forced switching design using controlled-smoking over 8 days, and (2) a 24-week follow-up (long-term phase) with unrestricted smoking under normal life conditions (Fig. 1). A sample size of seventy five (75) subjects per group was estimated to detect a 25% difference with 30% standard deviation in mean 24-hour urine NE and COHb between groups using a two-sided test, 5% type I error rate, and accounted for a dropout rate of less than 10% and would provide at least 80% power. This was based on data from a previously conducted clinical study in 11-mg tar cigarette smokers (Roethig et al., 2005). For the 24-week follow-up, we assumed that 90% of the subjects from the short-term phase would participate in the 24-week follow-up with a drop out rate of less than 15%.

2.4. Short-term phase

Subjects completed questionnaires at the beginning of the study to record smoking history and to determine each subject's score on the Fagerström Test for Nicotine Dependence (Fagerström, 1978). Subjects were admitted to one of the MDS Pharma Services clinical sites in Lincoln, NE or Phoenix, AZ for a 2-day pre-randomization period beginning on the evening of Day-3 and remained confined at the clinical site through Day 8 (Fig. 1). Controlled-smoking (Roethig et al., 2005; Roethig et al., 2007) was used to keep the maximum number of cigarettes smoked during the short-term phase by each subject as constant as possible. Before randomization, during the Acclimation day and the Baseline day, all subjects smoked MFF. They were monitored for cigarette consumption on Day-2 (Acclimation Day) to determine each subject's maximum daily allotment of cigarettes for the remainder of the short-term phase (Baseline through Day-8). Subjects were allowed to smoke up to their daily allotment of cigarettes in separate rooms designated for each study group from 0700 to 2300 at predetermined times only (every 32 min, based on the maximum of 30 cigarettes over 16 h) from Baseline through Day-8. Subjects were never forced to smoke and were allowed to smoke less than their daily allotment of cigarettes or even to quit smoking completely at any time during the study. Baseline exposure investigations were performed on Day-1. At the end of day 1, subjects were randomized into one of three groups: group MFF (control group) continued to smoke MFF cigarettes, group ML switched to ML cigarettes, group MUL switched to MUL cigarettes, for 8 days each. The groups were confined in separate rooms. Every cigarette smoked was documented and butts were collected from all subjects throughout the study to ensure compliance. Urine voided in each 24-h period was monitored by the clinical staff and collected for all subjects throughout the short-term phase. Morning blood samples were collected for cotinine and COHb between 0630 and 0700 under fasted conditions (no food or beverage except water during the preceding 8 h) before the first cigarette of the day. Evening blood samples were collected for cotinine and COHb at approximately 1900 (±10 min). Each subject smoked the first cigarette each morning and the first cigarette after lunch using the CReSSmicro™ device (Plowshare Ltd., Baltimore, MD) to obtain smoking topography data (number of puffs per cigarette, puff volume, puff duration, inter-puff interval).

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Table 1
FTC* smoke constituent yield (biomarkers of exposure)

	MFF		ML		MUL	
	Mean	SD	Mean	SD	Mean	SD
NNK [ng/cig] (total NNAL)	100.0 (n = 10)	5	87.9 (n = 10)	5.9	57.2 (n = 10)	5.0
Pyrene [ng/cig] (total 1-OHP)	35.6 (n = 10)	0.8	28.5 (n = 10)	0.7	17.4 (n = 10)	0.9
CO [mg/cig] (COHb)	12.9 (n = 5)	0.6	11.9 (n = 5)	0.6	7.12 (n = 5)	0.3
Acrolein [ug/cig] (3-HPMA)	63.6 (n = 2)	6.0	57.3 (n = 2)	5.7	27.3 (n = 2)	2.4
Benzene [ug/cig] (S-PMA)	39.7 (n = 10)	2.1	33.9 (n = 10)	1.7	26.4 (n = 5)	1.0

MFF = Full Flavor Marlboro, ML = Marlboro Lights, MUL = Marlboro Ultra Lights.
* FTC smoking machine conditions: 35 ml puff, 2 s puff duration, 1puff/min frequency; n represents the number of cigarettes per observation used for the smoking machines.

2.5. Long-term phase

Following the short-term phase all subjects were invited to participate in a 24-week follow-up with unrestricted smoking in their normal life settings, i.e. they could smoke at any time with no restrictions on daily cigarette numbers, but they had to continue to smoke the test product they had been randomized to in the short-term phase. Subjects in each study group received a supply of their respective test cigarettes at no cost. Biomarkers and topography were measured every 4 weeks during the long-term study using the same methods as in the short-term phase. However, in contrast to the short-term phase, all 24-h urine collections and topography measurements were ambulatory and only morning blood samples (between 0630 and 1100) were collected under fasting conditions, but the subjects could smoke before arriving at the clinic. Cigarette consumption was estimated by subtracting returned numbers of unsmoked cigarettes from dispensed numbers of cigarettes.

2.6. Bioanalytical methods

As described previously (Roethig et al., 2005), carboxyhemoglobin was measured in whole blood spectrophotometrically and urine mutagenicity (revertants/24 h) was analyzed with a modified Ames test (YG 1024 tester strain with metabolic activation) using concentrated aliquots from the 24-h urine sample. All other

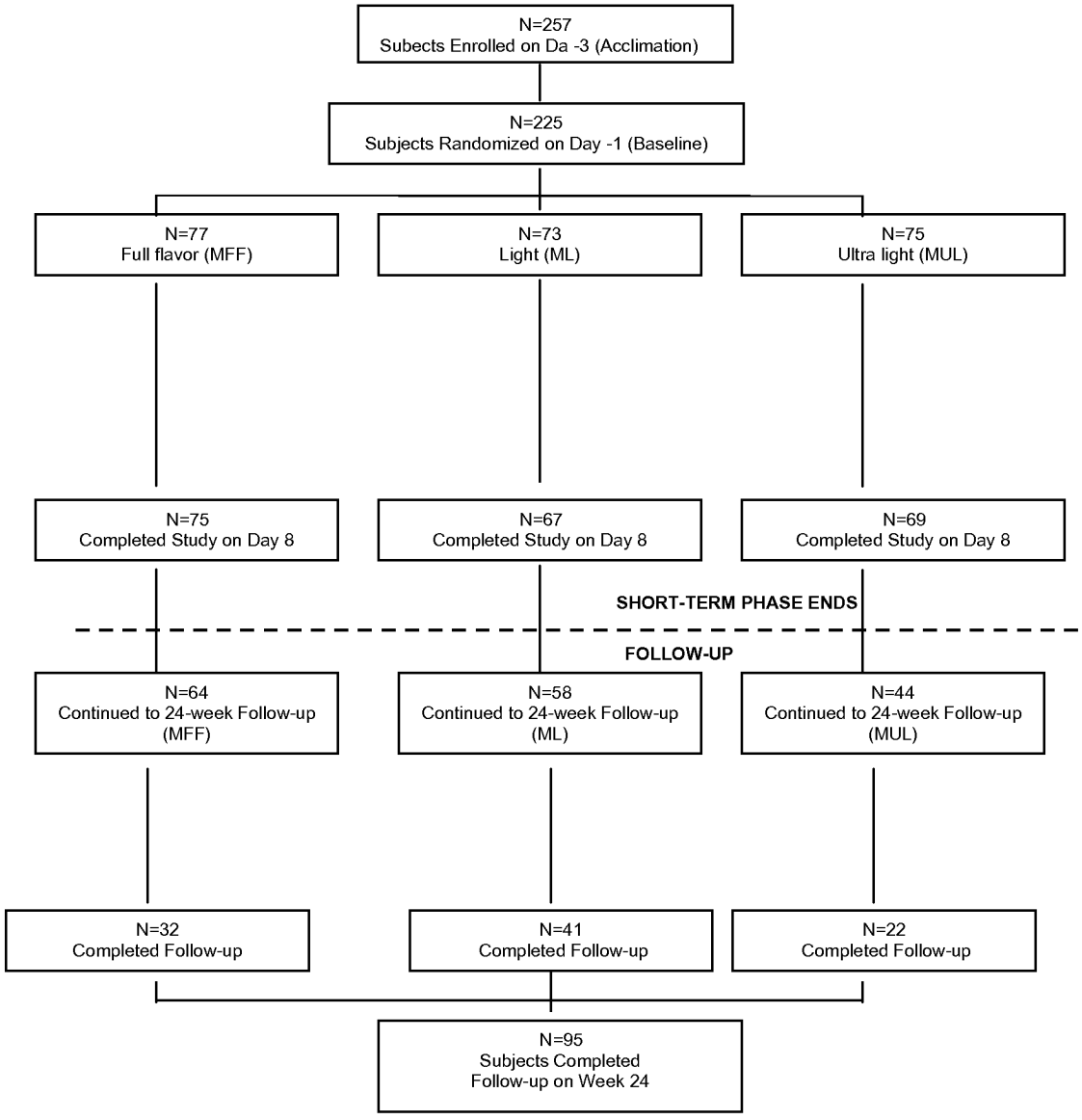


Fig. 1. Study design and subject disposition.

biomarkers were measured by liquid chromatography/tandem mass spectrometry (LC–MS/MS) methods validated according to the US Food and Drug Administration Guidance for Industry (US DHSS FDA, 2001). The LC–MS/MS method procedures and performances for urinary nicotine and metabolites, 1-OHP, 3-HPMA, *S*-PMA and plasma cotinine were described previously (Roethig et al., 2007). The method for urinary total NNAL was re-validated based on a previous procedure (Roethig et al., 2007) and had an improved sensitivity. The lower limit of quantitation for the total NNAL method used in this study was 5 pg/mL compared to 50 pg/mL reported previously. All analytical batches included appropriate calibration and quality control samples and met the acceptance criteria according to the FDA Guidance.

2.7. Data analysis

SAS® version 8.2 (SAS Institute, Inc.; Cary, North Carolina, USA) was used to carry out the statistical analysis. All randomized subjects in the short-term phase were included in the statistical analysis of the short-term phase. Subjects who agreed to participate in the long-term phase and had at least one post-Baseline measurement in the long-term phase were included in the statistical analysis of the long-term phase. Baseline values for both phases were calculated from Day 1 of the short-term phase (Fig. 1), but the populations in both phases were different based on the above mentioned specifications. Descriptive statistics were calculated for demographic characteristics, smoking history, and subject's score on the Fagerström Test for Nicotine Dependence at Baseline of the short-term phase. For the short-term phase, descriptive statistics were calculated for each biomarker and topography data by study group and study day and for the 24-h excretion of NE after adjusting for the number of cigarettes smoked per day. For the long-term phase, descriptive statistics were calculated for each biomarker and topography data by study group and study week. A linear mixed model for repeated-measures analysis of variance (ANOVA) was used to analyze biomarkers and topography data, with study group, study day or week, subject, and study group by study day or week interaction as the model terms. The interaction term was removed if it was not statistically significant ($p > 0.10$). All pair-wise comparisons between the study groups were performed using the values from all post-Baseline days or weeks. The Holm's step-down Bonferroni method for multiplicity adjustment (Westfall et al., 1999) was performed for NE and COHb in both the short- and long-term phases. Differences were considered statistically significant at $p \leqslant 0.05$. The overall mean was defined as the average of all post-Baseline data on all days and in all subjects of each group. Relative change from Baseline of the overall mean values was calculated using the following equation: [(overall post-Baseline mean − Baseline mean)/Baseline mean] × 100. During the short-term phase, the average post-Baseline inter-subject %CV of each group was the average of all inter-subject %CVs from Day 1 through Day 8.

3. Results

A total of 257 healthy adult male and female smokers with normal physical examinations, clinical laboratory tests, and ECGs volunteered and were enrolled in the study. Of these, 225 subjects were randomized into three study groups (77 in the MFF group; 73 in the ML group; and 75 in the MUL group), Fig. 1. Fourteen of these subjects discontinued for personal reasons. Of the 211 subjects (94%) who completed the short-term phase, 166 subjects (74%) consented to enroll into the 24-week follow-up (64 in the MFF group; 58 in the ML group; and 44 in the MUL group). At the end of this 24-week follow-up, 95 subjects (42%) completed the study (32 subjects completed in the MFF group, 41 in the ML group, and 22 in the MUL group) (Fig. 1). The most common reasons for drop-outs were non-compliance with the study procedures and loss to follow-up ($n = 38$), failed drug/alcohol testing ($n = 23$), failed clinical laboratory testing (e.g. liver enzymes >1.5 times the upper limit of normal) ($n = 4$), and personal reasons ($n = 6$). Due to the large drop-out in the 24-week follow-up, there was limited statistical power to perform comparisons between the study groups in the long-term phase.

3.1. Baseline characteristics

Table 2 summarizes the demographic and Baseline characteristics of the 225 subjects randomized to the three groups. There were no significant differences among the three groups in any of the mean Baseline variables including all biomarkers of exposure and smoking topography. Gender distributions were similar among the three groups with an almost 3:1 ratio of men:women in each group. At Baseline, mean values of subject's score on the Fagerström Test for Nicotine Dependence were between 5.4 and 5.8 on a scale of 0–9 and did not change during the study. Overall, 163 (72%) of the subjects had smoked MFF cigarettes for at least 10 years before enrolling in the study, and 62 (28%) had smoked MFF between 3 and 9 years.

3.2. MFF group

On average subjects smoked 19.7 cigarettes/day at Baseline. During the short-term phase, they smoked between 19.1 and 19.7 cigarettes/day and during the long-term phase increased their cigarette consumption from 19.7 to 26.3 cigarettes per day (+34%).

There was little change from Baseline to Day 8 in the mean NE/24 h urine excretion (Table 3a) during the short-term phase, with 47% of the subjects showing an increase and 53% a decrease (Table 3c). Overall, NE/24 h decreased in the long-term phase from 22.2 to 16.8 mg/24h (−24%) (Table 3b). NE/24 h was highly variable between subjects; the average post-Baseline inter-subject %CV in the short-term phase was 40.6%.

NE/cig decreased from 1.16 to 1.12 mg/cig in the short-term phase (−3%) and also showed large variability (Fig. 2).

In the short-term phase the overall post-Baseline levels were similar to those at Baseline for morning and evening plasma cotinine, total NNAL (Table 4a), total 1-OHP (Table 5a), urine mutagenicity (Table 6), morning and evening COHb (Tables 7a and 7b), *S*-PMA (Table 8a) and 3-HPMA. During the long-term phase, overall total NNAL levels decreased from 582 ng/24 h to 528 ng/24 h (−9%) (Table 4b), COHb increased from 3.9 to 4.7 %sat (+21%) (Table 7c) and 3-HPMA decreased from 2212 to 1919 μg/24 h (−13%). Levels of plasma cotinine, total 1-OHP (Table 5b), *S*-PMA (Table 8b) were similar to those at Baseline.

Number of puffs per cigarette, puff volume, puff duration and inter-puff interval did not change during the short-term phase and during the long-term phase.

Table 2
Subject demographic and Baseline characteristics[a]

Variable	MFF $N = 77$	ML $N = 73$	MUL $N = 75$	Overall $N = 225$
Age (years)				
Mean ± SD	36.5 ± 10.7	34.0 ± 8.9	34.4 ± 11.8	35.0 ± 10.5
(Min – max)	21 – 62	21 – 57	21 – 65	21 – 65
BMI (kg/m^2)				
Mean ± SD	26.1 ± 3.9	27.1 ± 4.6	26.6 ± 4.8	26.6 ± 4.4
(Min – max)	(19.6 – 38.4)	(20.2 – 40.1)	(19.3 – 36.5)	(19.3 – 40.1)
Race				
Black	1	3	1	5
Caucasian	69	64	67	200
Hispanic	7	4	6	17
Others	0	2	1	3
Gender				
Female	23	20	19	62
Male	54	53	56	163
Duration of smoking				
≤3 years	3	3	4	10
4–9 years	12	19	21	52
10–15 years	24	16	20	60
16–19 years	8	10	5	23
20–25 years	19	16	12	47
>25 years	11	9	13	33
Fagerström test for nicotine dependence				
Mean ± SD	5.4 ± 1.7	5.8 ± 1.8	5.5 ± 1.6	5.5 ± 1.6
(Min – max)	(1 – 9)	(1 – 9)	(0 – 9)	(0 – 9)
Number of cigs smoked at Baseline				
Mean ± SD	19.7 ± 4.2	20.3 ± 4.0	19.8 ± 4.2	
(Min – max)	(11 – 27)	(12 – 28)	(8 – 29)	

MFF = Full Flavor Marlboro, ML = Marlboro Lights, MUL = Marlboro Ultra Lights.
[a] At Day 2 (Acclimation) and Day 1 (Baseline), all subjects smoked MFF cigarettes.

Table 3a
Short-term phase: urine nicotine equivalents (NE) [mg/24 h]

	MFF mean ± SD	ML mean ± SD	MUL mean ± SD	MFF vs ML (p-value*)	MFF vs MUL (p-value*)
Baseline[a]	22.3 ± 8.1 (n = 74)	22.4 ± 7.3 (n = 69)	22.5 ± 9.4 (n = 73)		
Day 1	21.4 ± 10.5 (n = 74)	20.7 ± 6.6 (n = 70)	18.8 ± 6.9 (n = 73)	0.4589	0.0249
Day 2	23.1 ± 10.7 (n = 77)	19.2 ± 7.2 (n = 70)	17.2 ± 6.0 (n = 75)	0.0057	<.0001
Day 3	22.3 ± 8.4 (n = 76)	20.8 ± 8.1 (n = 76)	16.2 ± 6.3 (n = 74)	0.2959	<.0001
Day 4	21.5 ± 9.3 (n = 74)	19.3 ± 6.4 (n = 70)	15.8 ± 5.5 (n = 74)	0.0822	<.0001
Day 5	21.4 ± 8.0 (n = 73)	18.7 ± 7.3 (n = 68)	15.3 ± 6.7 (n = 73)	0.0357	<.0001
Day 6	21.1 ± 8.3 (n = 74)	18.0 ± 6.0 (n = 66)	15.1 ± 5.8 (n = 72)	0.0052	<.0001
Day 7	22.4 ± 8.2 (n = 74)	19.5 ± 6.8 (n = 66)	16.1 ± 6.2 (n = 70)	0.0221	<.0001
Day 8	20.8 ± 7.3 (n = 74)	20.1 ± 6.8 (n = 67)	16.4 ± 7.0 (n = 69)	0.4398#	0.0004#
Overall	21.8 ± 8.9 (n = 596)	19.6 ± 7.0 (n = 547)	16.4 ± 6.4 (n = 580)		

MFF = Full Flavor Marlboro, ML = Marlboro Lights, MUL = Marlboro Ultra Lights.
[a] At Baseline all subjects smoked MFF cigarettes.
p-values adjustments using Holm's step-down Bonferroni method for multiplicity were performed.
* p-values were derived from linear mixed model for repeated-measures ANOVA. Study group comparisons for each study day were performed when interaction term of study group by study day was significant ($p \leq 0.10$).

Table 3b
Long-term phase: urine nicotine equivalents (NE) [mg/24 h]

	MFF mean ± SD	ML mean ± SD	MUL mean ± SD	MFF vs ML (p-value*)	MFF vs MUL (p-value*)
Baseline[a]	22.2 ± 7.9 (n = 62)	22.1 ± 7.1 (n = 58)	23.0 ± 8.2 (n = 43)		
Week 0	21.8 ± 9.9 (n = 64)	20.5 ± 7.0 (n = 58)	16.9 ± 6.2 (n = 44)		
Week 4	16.18 ± 9.4 (n = 47)	15.5 ± 6.1 (n = 51)	13.7 ± 7.2 (n = 33)		
Week 8	14.0 ± 6.7 (n = 38)	15.6 ± 9.3 (n = 50)	12.0 ± 6.1 (n = 28)		
Week 12	14.1 ± 8.3 (n = 35)	13.5 ± 7.0 (n = 47)	11.5 ± 6.3 (n = 26)		
Week 16	15.6 ± 6.8 (n = 31)	13.5 ± 7.5 (n = 43)	11.6 ± 6.3 (n = 25)		
Week 20	16.1 ± 7.6 (n = 33)	12.8 ± 7.0 (n = 42)	10.2 ± 4.5 (n = 23)		
Week 24	16.0 ± 7.7 (n = 30)	14.6 ± 7.6 (n = 39)	12.6 ± 6.0 (n = 18)		
Overall	16.8 ± 8.8 (n = 278)	15.4 ± 7.8 (n = 330)	13.1 ± 6.5 (n = 197)	0.7627#	0.0203#

MFF = Full Flavor Marlboro, ML = Marlboro Lights, MUL = Marlboro Ultra Lights.
[a] At Baseline all subjects smoked MFF cigarettes.
p-values adjustments using Holm's step-down Bonferroni method for multiplicity were performed.
* p-values were derived from linear mixed model for repeated-measures ANOVA. Study group comparisons for overall values of post-Baseline study weeks were performed when interaction term of study group by study week was not significant ($p > 0.10$).

Table 10 summarizes the relative changes in all the measured biomarkers of exposure compared to Baseline.

3.3. ML group

On average subjects smoked 20.3 cigarettes/day at Baseline. During the short-term phase, they smoked between 20.0 and 20.5 cigarettes/day and during the long-term phase increased their cigarette consumption from 20.3 to 26.8 cigarettes per day (+32%).

Overall NE/24 h decreased from 22.4 to 19.6 mg/24 h during the short-term phase (−13%) (Table 3a). NE/24 h values were significantly lower than in the MFF control group ($p < 0.05$) on 4 of the 8 days. Seventy-three percent of the subjects showed a decrease of NE/24 h and 27% an increase on day 8 (Table 3c). Overall NE/24 h decreased during the long-term phase from 22.1 to 15.4 mg/24 h (−30%) (Table 3b). NE/24 h values were lower than in the MFF control group during the long-term phase, but the difference was not statistically significant ($p = 0.76$). NE/24 h was highly variable between subjects. The average post-Baseline inter-subject %CV in the short-term phase was 35.3%.

Table 3c
Change in nicotine equivalents daily excretion (mg/24 h) from Baseline[a] to Day 8

Study group	Nicotine equivalents decrease by			
	>0% to ≤20%	>20% to ≤40%	>40%	Total
MFF	19	11	8	38 (52.8%)
ML	26	19	3	48 (72.7%)
MUL	16	29	12	57 (85.1%)
	Nicotine equivalents increase by			
MFF	19	8	7	34 (47.2%)
ML	12	3	3	18 (27.3%)
MUL	6	2	2	10 (14.9%)

MFF = Full Flavor Marlboro, ML = Marlboro Lights, MUL = Marlboro Ultra Lights.
[a] At Baseline all subjects smoked MFF cigarettes.

NE/cig decreased from 1.1 at Baseline to 0.97 mg/cig in the short-term phase (−12%). It showed large variability (Fig. 2).

Plasma cotinine (morning) decreased slightly from 300 at Baseline to 281 ng/ml during the short-term phase (−6%) and from 302 to 276 ng/ml during the long-term phase (−9%). The values were lower than in the MFF group, but the difference was not statistically significant. Plasma cotinine (evening) decreased from 330 at Baseline to 296 ng/ml during the short-term phase (−10%).

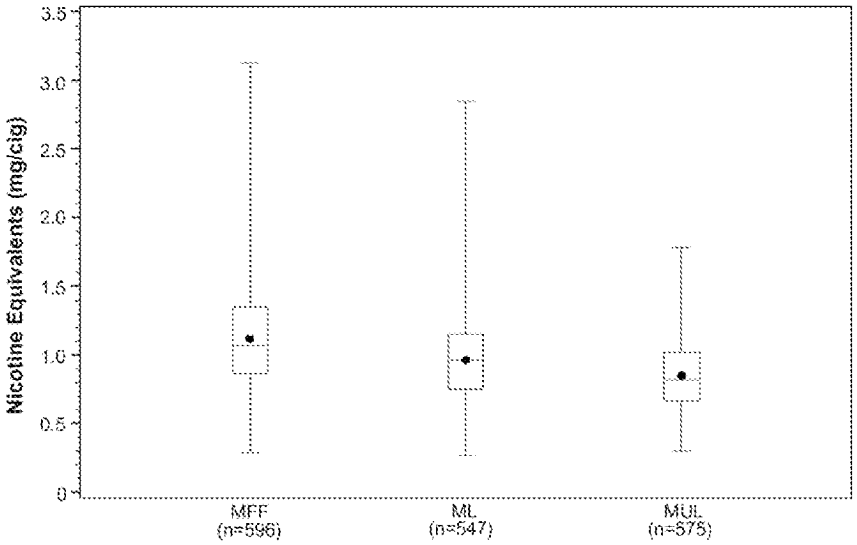
Total NNAL did not change during the short-term phase (+3%) but decreased from 629 to 509 ng/24 h during the long-term phase (−19%) (Tables 4a, 4b).

Total 1-OHP decreased from 233 at Baseline to 202 ng/24 h during the short-term phase (−13%) and from 237 to 229 ng/24 h during the long-term phase (−4%) (Tables 5a, 5b).

Urine mutagenicity (median) decreased from 21632 at Baseline to 20320 revertants/24 h during the short-term phase (−6%) (Table 6).

COHb (morning) was stable, whereas COHb (evening) increased from 6.9 at Baseline to 7.3%sat. during the short-term phase (+6%); COHb (morning) increased from 3.8 to 5.2%sat. during the long-term phase (+37%) (Tables 7a–7c).

S-PMA increased from 7.3 at Baseline to 7.9 μg/24 h during the short-term phase (+8%) and decreased from 7.3 to 6.3 μg/24 h during the long-term phase (−14%) (Tables 8a, 8b).



•,mean; *line in box,* median; *top* and *bottom bars of the box*, 25th to 75th percentile, respectively; *top bar* , maximum observation; *bottom bar,* minimum observation. p= 0.0005 for MFF vs ML; p<0.0001 for MFF vs MUL; n values represent the number of observations used to calculate the overall means. The overall mean is the average of all post-Baseline data on all days in all subjects of each group.

Fig. 2. Overall levels of nicotine equivalents adjusted for number of cigarettes smoked per day in 24-h urine of adult smokers of full flavor Marlboro, Marlboro Lights, and Marlboro Ultra Lights cigarettes in the short-term phase. •, mean; line in box, median; top and bottom bars of the box, 25th to 75th percentile, respectively; top bar, maximum observation; bottom bar, minimum observation. $p = 0.0005$ for MFF vs ML; $p < 0.0001$ for MFF vs MUL; n values represent the number of observations used to calculate the overall means. The overall mean is the average of all post-Baseline data on all days in all subjects of each group.

Table 4a
Short-term phase: urine total NNAL (ng/24 h)

	MFF mean ± SD	ML mean ± SD	MUL mean ± SD	MFF vs ML (p-value*)	MFF vs MUL (p-value*)
Baseline[a]	595.0 ± 279.9 (n = 74)	612.8 ± 245.9 (n = 70)	610.6 ± 332.6 (n = 74)		
Day 3	636.2 ± 305.7 (n = 76)	643.0 ± 292.8 (n = 70)	522.6 ± 240.8 (n = 74)		
Day 8	585.2 ± 267.4 (n = 74)	624.1 ± 280.1 (n = 67)	551.6 ± 269.2 (n = 69)		
Overall	611.2 ± 287.6 (n = 150)	633.8 ± 285.8 (n = 137)	536.6 ± 254.4 (n = 143)	.7056	.0496

MFF = Full Flavor Marlboro, ML = Marlboro Lights, MUL = Marlboro Ultra Lights.
[a] At Baseline all subjects smoked MFF cigarettes.
* p-values were derived from linear mixed model for repeated-measures ANOVA. Study group comparisons for overall values of post-Baseline study days were performed when interaction term of study group by study day was not significant ($p > 0.10$).

3-HPMA decreased from 2357 at Baseline to 2297 μg/24 h during the short-term phase (−3%) and decreased from 2424 to 1958 μg/24 h during the long-term phase(−19%).

Number of puffs per cigarette, puff volume, puff duration and inter-puff interval did not change significantly during the short-term phase; inter-puff interval decreased by 13% during the long-term phase but the other parameters were similar (Tables 9a, 9b).

Table 10 summarizes the relative changes in all the measured biomarkers of exposure compared to Baseline.

3.4. MUL group

On average subjects smoked 19.8 cigarettes/day at Baseline. During the short-term phase, they smoked between 18.6 and 19.8 cigarettes/day and during the long-term phase increased their cigarette consumption from 19.8 to 30.3 cigarettes per day (+53%).

NE/24 h decreased statistically significantly ($p < 0.0001$) from 22.5 at Baseline to 16.4 mg/24 h in the average Day 1–8 values in the short-term phase (−27%) (Table 3a). NE/24 h values were significantly lower than in the MFF control group ($p < 0.0001$) during the short-term phase. Eighty-five percent of the subjects showed a decrease of NE/24 h and 15% an increase on Day 8 (Table 3c). NE/24 h decreased in the long-term phase from 23 to 13.1 mg/24 h (−43%) (Table 3b). NE/24 h values were significantly lower than in the MFF control group ($p < 0.05$) in the long-term phase. NE/24 h was highly variable between subjects. The average post-Baseline inter-subject %CV in the short-term phase was 38.5%.

NE/cig decreased from 1.13 to 0.86 mg/cig in the short-term phase (−24%). It showed large variability (Fig. 2).

Plasma cotinine (morning) decreased from 295 at Baseline to 230 ng/ml during the short-term phase (−22%) and decreased from 302 to 237 ng/ml during the long-term phase (−22%). Cotinine levels were consistently and statistically significantly lower than in the MFF control group during the short-term phase ($p < 0.001$).

Total NNAL decreased from 610 to 536 ng/24 h during the short-term phase (−12%) and decreased from 600 to 424 ng/24 h during the long-term phase (−29%) (Tables 4a, 4b). Total NNAL levels were statistically significantly lower than in the MFF control group during the short-term phase ($p = 0.0496$).

Total 1-OHP decreased from 221 to 179 ng/24 h during the short-term phase (−19%) and from 222 to 208 ng/24 h during the long-term phase (−6%) (Tables 5a, 5b). Total 1-OHP levels were statistically significantly lower than in the MFF control group during the short-term and the long-term phase ($p < 0.05$).

Table 4b
Long-term phase: urine total NNAL (ng/24 h)

	MFF mean ± SD	ML mean ± SD	MUL mean ± SD	MFF vs ML (p-value*)	MFF vs MUL (p-value*)
Baseline[a]	582.4 ± 270.0 (n = 62)	628.7 ± 250.3 (n = 58)	600.1 ± 251.1 (n = 44)		
Week 0	627.2 ± 345.3 (n = 64)	636.0 ± 292.8 (n = 58)	556.4 ± 246.4 (n = 44)		
Week 4	543.5 ± 358.9 (n = 47)	554.2 ± 245.2 (n = 51)	437.7 ± 240.5 (n = 33)		
Week 8	477.4 ± 281.2 (n = 37)	519.7 ± 297.5 (n = 50)	392.8 ± 214.8 (n = 28)		
Week 12	432.4 ± 274.5 (n = 35)	471.5 ± 274.3 (n = 47)	378.0 ± 257.7 (n = 26)		
Week 16	487.8 ± 280.9 (n = 32)	437.5 ± 275.1 (n = 43)	367.0 ± 225.4 (n = 25)		
Week 20	493.5 ± 274.9 (n = 32)	399.7 ± 257.6 (n = 42)	314.5 ± 176.6 (n = 23)		
Week 24	542.0 ± 348.1 (n = 30)	486.3 ± 247.4 (n = 39)	408.8 ± 253.4 (n = 18)		
Overall	527.6 ± 320.2 (n = 277)	508.7 ± 279.9 (n = 330)	424.0 ± 243.1 (n = 197)	.7169	.0831

MFF = Full Flavor Marlboro, ML = Marlboro Lights, MUL = Marlboro Ultra Lights.
[a] At Baseline all subjects smoked MFF cigarettes.
* p-values were derived from linear mixed model for repeated-measures ANOVA. Study group comparisons for overall values of post-Baseline study weeks were performed when interaction term of study group by study week was not significant ($p > 0.10$).

Table 5a
Short-term phase: urine total 1-OHP daily (ng/24 h)

	MFF mean ± SD	ML mean ± SD	MUL mean ± SD	MFF vs ML (p-value*)	MFF vs MUL (p-value*)
Baseline[a]	232.8 ± 114.3 (n = 72)	232.9 ± 134.8 (n = 70)	220.8 ± 105.4 (n = 74)		
Day 1	222.2 ± 123.6 (n = 73)	199.8 ± 95.1 (n = 70)	175.6 ± 73.3 (n = 73)	.1168	.0020
Day 2	216.5 ± 102.6 (n = 76)	187.3 ± 91.6 (n = 69)	176.7 ± 70.5 (n = 75)	.0460	.0087
Day 3	215.0 ± 10.1 (n = 76)	204.2 ± 124.4 (n = 70)	160.1 ± 79.1 (n = 74)	.5443	.0013
Day 4	199.0 ± 84.5 (n = 73)	191.5 ± 105.3 (n = 70)	167.0 ± 79.0 (n = 74)	.6812	.0362
Day 5	285.3 ± 154.0 (n = 74)	239.8 ± 137.1 (n = 68)	230.8 ± 132.4 (n = 73)	.0739	.0231
Day 6	237.3 ± 125.7 (n = 75)	204.9 ± 89.9 (n = 66)	185.5 ± 79.0 (n = 72)	.0741	.0016
Day 7	237.3 ± 124.7 (n = 74)	199.9 ± 95.4 (n = 66)	175.3 ± 74.7 (n = 70)	.0344	.0002
Day 8	185.4 ± 95.5 (n = 74)	186.9 ± 91.1 (n = 67)	157.3 ± 74.2 (n = 69)	.8138	.0544
Overall	224.8 ± 119.5 (n = 595)	201.8 ± 105.7 (n = 546)	178.6 ± 87.2 (n = 580)		

MFF = Full Flavor Marlboro, ML = Marlboro Lights, MUL = Marlboro Ultra Lights.
[a] At Baseline all subjects smoked MFF cigarettes.
* p-values were derived from linear mixed model for repeated-measures ANOVA. Study group comparisons for each study day were performed when interaction term of study group by study day was significant ($p \leqslant 0.10$).

Table 5b
Long-term phase: urine total 1-OHP daily (ng/24 h)

	MFF mean ± SD	ML mean ± SD	MUL mean ± SD	MFF vs ML (p-value*)	MFF vs MUL (p-value*)
Baseline[a]	232.1 ± 108.9 (n = 60)	237.3 ± 139.5 (n = 58)	221.7 ± 106.6 (n = 44)		
Week 0	192.92 ± 99.0 (n = 64)	188.0 ± 93.3 (n = 58)	157.9 ± 73.3 (n = 44)		
Week 4	243.6 ± 153.0 (n = 47)	244.1 ± 232.4 (n = 51)	359.2 ± 639.6 (n = 33)		
Week 8	217.3 ± 140.7 (n = 37)	281.9 ± 231.2 (n = 50)	185.0 ± 102.4 (n = 28)		
Week 12	235.9 ± 277.4 (n = 35)	224.7 ± 154.1 (n = 47)	199.1 ± 136.3 (n = 26)		
Week 16	228.9 ± 168.1 (n = 32)	197.9 ± 125.6 (n = 43)	177.7 ± 97.0 (n = 25)		
Week 20	222.2 ± 143.6 (n = 33)	218.7 ± 189.8 (n = 42)	161.2 ± 103.5 (n = 23)		
Week 24	315.8 ± 307.2 (n = 30)	245.0 ± 150.8 (n = 39)	199.3 ± 88.6 (n = 19)		
Overall	231.0 ± 186.4 (n = 278)	228.1 ± 176.2 (n = 330)	207.5 ± 281.7 (n = 198)	.8493	.0088

MFF = Full Flavor Marlboro, ML = Marlboro Lights, MUL = Marlboro Ultra Lights.
[a] At Baseline all subjects smoked MFF cigarettes.
* p-values were derived from linear mixed model for repeated-measures ANOVA. Study group comparisons for overall values of post-Baseline study weeks were performed when interaction term of study group by study week was not significant ($p > 0.10$).

Table 6
Short-term phase: urine mutagenicity (revertants/24 h)

	MFF	ML	MUL	MFF vs ML (p-value*)	MFF vs MUL (p-value*)
Baseline[a]					
Mean ± SD	24878 ± 18928	27048 ± 21639	23271 ± 12272		
Median	19656	21632	22971		
n	75	73	74		
Day 8					
Mean ± SD	23352 ± 14734	23950 ± 13203	19499 ± 12071	0.6954	0.066
Median	20726	20320	17791		
n	73	67	68		

MFF = Full Flavor Marlboro, ML = Marlboro Lights, MUL = Marlboro Ultra Lights.
[a] At Baseline all subjects smoked MFF cigarettes.
* p-values were derived from linear mixed model of ANOVA. Square root transformation of data was performed for study group comparisons.

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Table 7a
Short-term phase: COHb (morning) [% sat]

	MFF mean ± SD	ML mean ± SD	MUL mean ± SD	MFF vs ML (p-value*)	MFF vs MUL (p-value*)
Baseline[a]	3.9 ± 1.1 (n = 77)	3.8 ± 0.9 (n = 73)	3.9 ± 1.0 (n = 75)		
Day 1	3.3 ± 0.8 (n = 75)	3.5 ± 0.8 (n = 73)	3.5 ± 0.9 (n = 75)	.2404	.2598
Day 2	3.7 ± .8 (n = 77)	4.0 ± 0.9 (n = 71)	3.5 ± 0.8 (n = 75)	.0085	.1176
Day 3	3.8 ± 0.8 (n = 77)	4.0 ± 0.9 (n = 70)	3.4 ± 0.7 (n = 74)	.0559	.0040
Day 4	3.7 ± 0.8 (n = 75)	3.8 ± 0.8 (n = 70)	3.3 ± 0.7 (n = 75)	.7586	.0009
Day 5	3.8 ± 0.8 (n = 74)	3.9 ± 0.8 (n = 70)	3.3 ± 0.8 (n = 74)	.7611	.0001
Day 6	3.5 ± 0.7 (n = 75)	3.8 ± 0.7 (n = 69)	3.1 ± 0.7 (n = 73)	.0303	.0032
Day 7	3.5 ± 0.8 (n = 74)	3.8 ± 0.8 (n = 67)	3.3 ± 0.9 (n = 71)	.0409	.0517
Day 8	3.5 ± 0.7 (n = 75)	4.0 ± 0.8 (n = 65)	3.2 ± 0.7 (n = 70)	.0020[#]	.0614[#]
Overall	3.6 ± 0.8 (n = 602)	3.8 ± 0.8 (n = 555)	3.3 ± 0.8 (n = 587)		

MFF = Full Flavor Marlboro, ML = Marlboro Lights, MUL = Marlboro Ultra Lights.
[a] At Baseline all subjects smoked MFF cigarettes.
[#] p-values adjustments using Holm's step-down Bonferroni method for multiplicity were performed.
[*] p-values were derived from linear mixed model for repeated-measures ANOVA. Study group comparisons for each study day were performed when interaction term of study group by study day was significant ($p \leqslant 0.10$).

Table 7b
Short-term phase: COHb (evening) [% sat]

	MFF mean ± SD	ML mean ± SD	MUL mean ± SD	MFF vs ML (p-value*)	MFF vs MUL (p-value*)
Baseline[a]	6.9 ± 1.8 (n = 77)	6.9 ± 1.5 (n = 73)	6.9 ± 1.9 (n = 75)		
Day 1	7.0 ± 1.7 (n = 77)	7.3 ± 1.6 (n = 71)	6.1 ± 1.4 (n = 75)	0.3038	0.0010
Day 2	7.0 ± 1.7 (n = 77)	7.0 ± 1.6 (n = 70)	6.2 ± 1.6 (n = 75)	0.8453	0.0012
Day 3	7.0 ± 1.7 (n = 76)	7.5 ± 1.6 (n = 70)	6.2 ± 1.6 (n = 75)	0.0344	0.0038
Day 4	6.9 ± 1.6 (n = 75)	7.1 ± 1.7 (n = 70)	6.0 ± 1.7 (n = 74)	0.4569	0.0004
Day 5	6.8 ± 1.6 (n = 75)	7.3 ± 1.7 (n = 70)	5.9 ± 1.8 (n = 73)	0.0563	0.0009
Day 6	6.8 ± 1.7 (n = 74)	7.3 ± 1.5 (n = 68)	6.1 ± 1.6 (n = 74)	0.1051	0.0078
Day 7	6.9 ± 1.6 (n = 75)	7.3 ± 1.5 (n = 67)	5.8 ± 1.6 (n = 72)	0.1701	<.0001
Day 8	6.4 ± 1.4 (n = 75)	7.4 ± 1.5 (n = 67)	6.0 ± 1.7 (n = 69)	0.0005[#]	0.1251[#]
Overall	6.9 ± 1.6 (n = 604)	7.3 ± 1.6 (n = 553)	6.0 ± 1.6 (n = 587)		

MFF = Full Flavor Marlboro, ML = Marlboro Lights, MUL = Marlboro Ultra Lights.
[a] At Baseline all subjects smoked MFF cigarettes.
[#] p-values adjustments using Holm's step-down Bonferroni method for multiplicity were performed.
[*] p-values were derived from linear mixed model for repeated-measures ANOVA. Study group comparisons for each study day were performed when interaction term of study group by study day was significant ($p \leqslant 0.10$).

Table 7c
Long-term Phase: COHb (Morning) [% sat]

	MFF Mean ± SD	ML mean ± SD	MUL mean ± SD	MFF vs ML (p-value*)	MFF vs MUL (p-value*)
Baseline[a]	3.9 ± 1.1 (n = 64)	3.8 ± 1.0 (n = 58)	4.0 ± 1.1 (n = 44)		
Week 0	3.5 ± 0.7 (n = 64)	4.0 ± 0.8 (n = 57)	3.3 ± 0.8 (n = 44)		
Week 4	5.0 ± 2.1 (n = 48)	5.4 ± 1.9 (n = 51)	4.7 ± 1.6 (n = 35)		
Week 8	4.8 ± 1.6 (n = 43)	5.1 ± 1.7 (n = 49)	4.9 ± 1.5 (n = 30)		
Week 12	4.8 ± 1.6 (n = 37)	5.2 ± 1.8 (n = 49)	4.9 ± 1.4 (n = 29)		
Week 16	5.1 ± 1.5 (n = 35)	5.3 ± 2.2 (n = 46)	5.0 ± 1.7 (n = 27)		
Week 20	5.4 ± 1.8 (n = 34)	6.0 ± 1.8 (n = 41)	5.3 ± 2.1 (n = 24)		
Week 24	5.5 ± 1.9 (n = 32)	5.7 ± 2.2 (n = 41)	5.1 ± 1.9 (n = 22)		
Overall	4.7 ± 1.7 (n = 293)	5.2 ± 1.9 (n = 334)	4.6 ± 1.7 (n = 211)	.0019[#]	.2130[#]

MFF = Full Flavor Marlboro, ML = Marlboro Lights, MUL = Marlboro Ultra Lights.
[a] At Baseline all subjects smoked MFF cigarettes.
[#] p-values adjustments using Holm's step-down Bonferroni method for multiplicity were performed.
[*] p-values were derived from linear mixed model for repeated-measures ANOVA. Study group comparisons for overall values of post-Baseline study weeks were performed when interaction term of study group by study week was not significant ($p \leqslant 0.10$).

Urine mutagenicity (median) decreased from 22971 to 17791 revertants/24 h during the short-term phase (−23%) (Table 6).

COHb (morning) decreased from 3.9 to 3.3 %sat. during the short-term phase (−15%) and increased from 4.0 to 4.6 %sat. during the long-term phase (+15%) (Tables 7a, 7c, 7b). COHb (evening) decreased from 6.9 at Baseline to 6.0 %sat during the short-term phase (−13%).

S-PMA decreased from 6.3 to 6.2 μg/24 h during the short-term phase (−2%) and decreased from 5.9 to 4.7 μg/24 h during the long-term phase (−20%) (Tables 8a, 8b).

3-HPMA decreased from 2279 to 2090 μg/24 h during the short-term phase (−8%) and decreased from 2363 to 1858 μg/24 h during the long-term phase (−21%).

Number of puffs per cigarette, puff volume, and puff duration did not change significantly during the short-term phase and during the long-term phase. Inter-puff interval decreased from 28 to 21 sec. during the long-term phase (−24%); values were statistically significantly lower than in the MFF control group (Tables 9a, 9b).

Table 10 summarizes the relative changes in all the measured biomarkers of exposure compared to Baseline.

3.5. Safety

There were no serious adverse events in the study and no clinically relevant adverse events reported in any of the groups both the short and the long-term phases.

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Table 8a
Short-term phase: urine *S*-PMA (μg/24 h)

	MFF mean ± SD	ML mean ± SD	MUL mean ± SD	MFF vs ML (*p*-value*)	MFF vs MUL (*p*-value*)
Baseline[a]	7.2 ± 4.0 (*n* = 74)	7.3 ± 4.4 (*n* = 72)	6.3 ± 4.0 (*n* = 74)		
Day 1	7.4 ± 4.9 (*n* = 74)	7.6 ± 4.3 (*n* = 71)	5.9 ± 3.6 (*n* = 73)	.7581	.0258
Day 2	7.4 ± 4.1 (*n* = 76)	7.6 ± 4.5 (*n* = 70)	6.5 ± 4.3 (*n* = 75)	.8017	.2043
Day 3	7.6 ± 4.1 (*n* = 76)	8.7 ± 5.6 (*n* = 70)	6.1 ± 3.7 (*n* = 74)	.1263	.0350
Day 4	6.9 ± 3.5 (*n* = 74)	7.7 ± 5.0 (*n* = 70)	6.0 ± 3.5 (*n* = 74)	.2107	.1582
Day 5	7.5 ± 4.3 (*n* = 75)	7.6 ± 5.3 (*n* = 68)	7.1 ± 7.0 (*n* = 73)	.8630	.6318
Day 6	7.9 ± 4.4 (*n* = 75)	8.1 ± 4.8 (*n* = 66)	6.4 ± 3.9 (*n* = 72)	.6487	.0822
Day 7	7.3 ± 4.4 (*n* = 74)	7.9 ± 4.9 (*n* = 66)	5.9 ± 3.6 (*n* = 70)	.3820	.0997
Day 8	6.9 ± 3.9 (*n* = 74)	8.1 ± 5.0 (*n* = 67)	6.2 ± 4.0 (*n* = 69)	.0901	.4494
Overall	7.4 ± 4.2 (*n* = 598)	7.9 ± 4.9 (*n* = 548)	6.2 ± 4.3 (*n* = 580)		

MFF = Full Flavor Marlboro, ML = Marlboro Lights, MUL = Marlboro Ultra Lights.
[a] At Baseline all subjects smoked MFF cigarettes.
* *p*-values were derived from linear mixed model for repeated-measures ANOVA. Study group comparisons for each study day were performed when interaction term of study group by study day was significant ($p \leqslant 0.10$).

Table 8b
Long-term phase: urine *S*-PMA (μg/24 h)

	MFF mean ± SD	ML mean ± SD	MUL mean ± SD	MFF vs ML (*p*-value*)	MFF vs MUL (*p*-value*)
Baseline[a]	7.2 ± 4.0 (*n* = 62)	7.3 ± 4.2 (*n* = 57)	5.9 ± 3.6 (*n* = 44)		
Week 0	7.3 ± 4.4 (*n* = 64)	8.1 ± 5.0 (*n* = 58)	6.1 ± 3.9 (*n* = 44)		
Week 4	6.1 ± 4.3 (*n* = 47)	6.6 ± 4.0 (*n* = 51)	4.9 ± 3.2 (*n* = 33)		
Week 8	5.8 ± 5.0 (*n* = 37)	6.7 ± 5.2 (*n* = 50)	4.1 ± 2.5 (*n* = 28)		
Week 12	5.9 ± 3.4 (*n* = 35)	6.0 ± 4.3 (*n* = 47)	4.3 ± 3.7 (*n* = 26)		
Week 16	6.6 ± 4.5 (*n* = 32)	5.6 ± 4.2 (*n* = 43)	4.6 ± 3.5 (*n* = 25)		
Week 20	5.5 ± 3.9 (*n* = 33)	4.7 ± 3.4 (*n* = 42)	3.4 ± 2.2 (*n* = 23)		
Week 24	5.8 ± 4.3 (*n* = 30)	5.4 ± 4.1 (*n* = 39)	4.0 ± 2.4 (*n* = 19)		
Overall	6.3 ± 4.3 (*n* = 278)	6.3 ± 4.5 (*n* = 330)	4.7 ± 3.3 (*n* = 198)	.5269	.0496

MFF = Full Flavor Marlboro, ML = Marlboro Lights, MUL = Marlboro Ultra Lights.
[a] At Baseline all subjects smoked MFF cigarettes.
* *p*-values were derived from linear mixed model for repeated-measures ANOVA. Study group comparisons for overall values of post-Baseline study weeks were performed when interaction term of study group by study week was not significant ($p \leqslant 0.10$).

Table 9a
Short-term phase: inter-puff linterval (s)

	MFF mean ± SD	ML mean ± SD	MUL mean ± SD	MFF vs ML (*p*-value*)	MFF vs MUL (*p*-value*)
Baseline[a]	31.1 ± 8.6 (*n* = 73)	28.9 ± 7.4 (*n* = 70)	29.9 ± 8.4 (*n* = 71)		
Day 1	33.2 ± 10.8 (*n* = 76)	28.7 ± 8.1 (*n* = 66)	28.5 ± 9.2 (*n* = 68)	.0129	.0043
Day 2	34.2 ± 11.9 (*n* = 66)	29.1 ± 8.4 (*n* = 66)	28.4 ± 9.3 (*n* = 66)	.0064	.0025
Day 3	33.2 ± 9.8 (*n* = 73)	29.5 ± 9.0 (*n* = 67)	28.6 ± 10.2 (*n* = 71)	.0145	.0047
Day 4	30.9 ± 9.5 (*n* = 73)	30.7 ± 9.5 (*n* = 64)	28.4 ± 9.0 (*n* = 72)	.9507	.0551
Day 5	31.6 ± 10.7 (*n* = 73)	28.8 ± 6.7 (*n* = 67)	28.5 ± 9.1 (*n* = 69)	.0595	.0593
Day 6	31.2 ± 9.9 (*n* = 73)	29.7 ± 9.2 (*n* = 66)	29.5 ± 9.9 (*n* = 71)	.4240	.1896
Day 7	33.6 ± 11.5 (*n* = 73)	31.3 ± 9.8 (*n* = 64)	29.0 ± 8.1 (*n* = 69)	.1923	.0050
Day 8	33.5 ± 10.9 (*n* = 73)	28.3 ± 7.6 (*n* = 64)	28.2 ± 8.6 (*n* = 66)	.0022	.0016
Overall	32.7 ± 10.6 (*n* = 580)	29.5 ± 8.6 (*n* = 524)	28.6 ± 9.2 (*n* = 552)		

MFF = Full Flavor Marlboro, ML = Marlboro Lights, MUL = Marlboro Ultra Lights.
[a] At Baseline all subjects smoked MFF cigarettes.
* *p*-values were derived from linear mixed model for repeated-measures ANOVA. Study group comparisons for each study day were performed when interaction term of study group by study day was significant ($p \leqslant 0.10$).

4. Discussion

The present study evaluated the effects of switching from MFF (a 15-mg tar (FTC) cigarette) to the lower tar cigarettes ML (11-mg tar) or MUL (6-mg tar) compared to continuing to smoke MFF. There has been a long lasting controversial discussion regarding the study designs of exposure studies in smokers (Benowitz, 2001).While in highly controlled studies accurate data is generated, the question is whether the results are predictable for smokers in their normal environment. On the other hand, studies in smokers in their normal environment have limitations concerning the accuracy of the data collected. Based on our experience in evaluating different cigarette designs (Roethig et al., 2005; Roethig et al., 2007; Sarkar et al., 2004), we applied the new approach to address these issues. The strength of this study obviously lies in the randomized, controlled, short- and long-term design, the adequately large sample size (225 subjects), and the rich array of biomarkers investigated.

While the short-term phase was successfully completed according to the protocol, there were difficulties in the 24-week follow-up phase. Fewer subjects than expected consented to continue in the long-term follow-up, and in the MFF and MUL groups 50% of the subjects dropped out, thus limiting the power for statistical testing in the long-term phase (Fig. 1). During the long-term phase cigarette consumption—measured as number of cigarettes dispensed and returned—increased between 32 and 53% in all three groups, whereas the NE excretion decreased by 24–43%. While in the short-term phase, every smoked cigarette was accurately ac-

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Table 9b
Long-term phase: inter-puff interval (s)

	MFF mean ± SD	ML mean ± SD	MUL mean ± SD	MFF vs ML (p-value*)	MFF vs MUL (p-value*)
Baseline[a]	31.2 ± 8.3 (n = 60)	28.5 ± 7.3 (n = 57)	28.2 ± 8.6 (n = 40)		
Overall	27.1 ± 9.6 (n = 150)	24.8 ± 9.7 (n = 202)	21.3 ± 7.7 (n = 118)	.2727	.0074

MFF = Full Flavor Marlboro, ML = Marlboro Lights, MUL = Marlboro Ultra Lights.
[a] At Baseline all subjects smoked MFF cigarettes.
* p-values were derived from linear mixed model for repeated-measures ANOVA. Study group comparisons for overall values of post-Baseline study weeks were performed when interaction term of study group by study week was not significant ($p \leqslant 0.10$).

Table 10
Relative change of overall mean values from Baseline[a] (%)

Biomarker	MFF		ML		MUL	
	s.t.	l.t.	s.t.	l.t.	s.t.	l.t.
Number of cigarettes	−2	+34	0	+32	−4.0	+53
NE/24 h	−2	−24	−13*	−30	−27*	−43*
NE/cig	−3		−12*		−24*	
Cotinine, morning	0	−3	−6	−9	−22*	−22
Cotinine, evening	−1		−10		−25*	
Total NNAL	+3	−9	+3	−19	−12*	−29
Total 1-OHP	−3	0	−13	−4	−19*	−6*
Urine mutagenicity	+5		−6		−23	
COHb, morning	−8	+21	0	+37	−15	+15
COHb, evening	0		+6		−13	
S-PMA	+3	−13	+8	−14	−2	−20*
3-HPMA	−1	−13	−3	−19	−8	−21
Inter-Puff Interval	5	−13	2	−13	−4	−24*

MFF = Full Flavor Marlboro, ML = Marlboro Lights, MUL = Marlboro Ultra Lights.
s.t. = short-term phase; l.t. = long-term phase.
Overall means: average of all post-Baseline data on all days in all subjects of each group.
[a] At Baseline all subjects smoked MFF cigarettes.
* $p < 0.05$ for comparing to MFF.

counted for by the clinical staff, the numbers derived from dispensed and returned cigarettes during the follow-up phase are, at best, a rough estimate of the cigarettes smoked.

The statistically significant reductions in 24-h NE after switching from MFF to ML (−13%) and MUL (− 27%) in the short-term, controlled-smoking phase appear to have continued into the follow-up phase (ML −30%, and MUL −43% (Table 10)). This suggests that the results from controlled, short-term phase studies are predictive for smokers in their normal environment and is supported by previously published data (Sarkar et al., 2004). The larger decrease in 24-h NE seen in all three groups of the long-term phase cannot be explained by incomplete urine collections, since the 24-h urine creatinine excretions were comparable to those of the short-term phase (data not shown). A plausible explanation for these reductions in 24-h NE is that smokers in their normal environment have fewer opportunities to smoke. We have observed similar effects in other studies (Frost-Pineda et al., 2008).

Like with the 24-h nicotine reductions, switching from MFF to ML or MUL also showed statistically significant nicotine per cigarette reductions in the controlled short-term phase, indicating that switching to lower tar (FTC) cigarettes on average decreases the exposure to nicotine. Interestingly, the nicotine exposure per cigarette, i.e. mean nicotine uptake per cigarette in the ML group after switching from MFF was similar to values found in smokers who had smoked ML as their regular brand (Roethig et al., 2007). These results support the validity of forced switching studies to evaluate exposure to different cigarette products. Based on the differences in FTC nicotine yield of the three test cigarettes, the machine-generated yields were reduced by 27% and 45% when comparing MFF to ML and MUL cigarettes, respectively. In our study the overall reductions in NE/24 h were 13% and 27%, respectively, suggesting "partial compensation". Scherer (1999) reviewed a number of studies in the literature and concluded that smokers "partially compensate for a different smoke yield" when switching to cigarettes with different machine-generated yields. The results of our study are consistent with this conclusion. Scherer also concluded that the change in puff volume is the most probable mechanism responsible for partial compensation in smokers who switch to lower tar cigarettes, while changing the number of cigarettes does not appear to be a common mechanism. Our results show no changes in puff volume but statistically significant changes in inter-puff interval, especially when switching from MFF to MUL, suggesting that inter-puff interval may play a role in compensatory behavioral changes.

All particulate phase biomarkers investigated in this study appeared to follow the same pattern as NE, but the relative reductions for the other biomarkers were smaller (−2 to −25%). This is not unexpected, as exposure to 1-OHP for example is also influenced by other sources than cigarette smoke (e.g. grilled meat). For a biomarker with a long elimination half-life like total NNAL (Hecht et al., 1999), since steady-state is not reached after 8 days, the full effect of switching could only be observed in the 24-week follow-up phase. Biomarkers of exposure to smoke constituents of the gas/vapor phase were reduced to a lesser extent when switching to MUL (−2 to −15%) in the short-term phase. These results are consistent with other published results that show COHb decreases when subjects switch to low-tar products (Russell et al., 1973). The increase in COHb in this group (like in the other two groups) during the 24-week follow-up is however an artifact of different measuring times in the short- and long-term phases. Study logistics required that blood be collected later in the mornings during the 24-week follow-up. Overall the data indicate that switching from MFF to MUL results in a reduction in exposure to particulate phase cigarette smoke constituents by about 25% while gas/vapor phase smoke constituents are reduced to a lesser extent.

Morning COHb did not change in the short-term phase, but evening COHb increased by 6% in the ML group compared to the MFF group, although this change did not reach statistical significance. This trend persisted into the 24-week follow-up with COHb values most of the time being higher than in the MFF control group. These results are similar to those reported by Benowitz et al., 1982). Compared to the MFF cigarette, the ML cigarette delivers FTC machine-generated smoke with a higher CO/tar ratio, in part, because of its greater filter efficiency for tar and nicotine than the MFF cigarette. Evening COHb was measured in the short-term study because sampling COHb at the end of a smoking day is a good indicator of the daily CO exposure (Hee et al., 1995; Roethig et al., 2005). The other two gas-vapor-phase markers, 3-HPMA and *S*-PMA were not much different from the control MFF group.

The inter-subject variability observed for all biomarkers in all groups was remarkably high despite a very high level of standardization in the short-term phase. As can be seen in Fig. 2 the maximum nicotine exposure from one cigarette is 6–10 times higher than the minimum nicotine exposure. Under less standardized conditions, i.e. normal life setting, this range is expected to be even larger. The inter-subject variability for tobacco-specific biomarkers can arise from differences in how individuals smoke cigarettes and

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how they metabolize tobacco constituents (Benowitz, 2001). For biomarkers which are not specific for tobacco smoke, such as 1-OHP, 3-HPMA, *S*-PMA, COHb and urine mutagenicity, diet and environmental factors contribute to a major extent to the variability in exposure to polycyclic aromatic hydrocarbons, acrolein, CO, benzene and mutagenic substances (Feng et al., 2006).

This study indicates that switching to lower tar cigarettes on average leads to a measurable reduction in nicotine and other biomarkers considered surrogates of tar exposure.

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